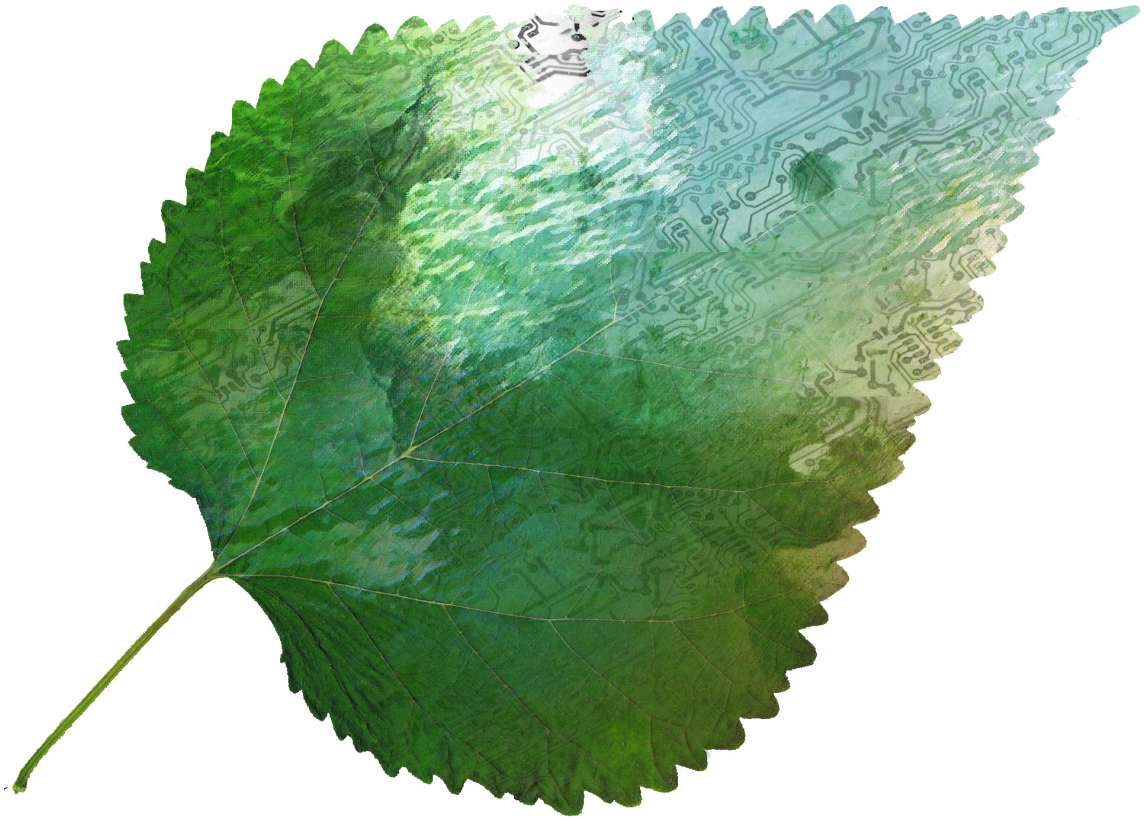
Introduction to Opportunities in Plant Synthetic Biology



21-22 May 2013
University of Nottingham

GARNet

A GARNet meeting

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New Phytologist

Journal of Experimental Botany
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An Introduction to Opportunites in Plant Synthetic Biology

University of Nottingham, UK

Organising Committee

Charis Cook (GARNet)
Ruth Bastow (GARNet)
Susie Lydon (University of Nottingham)
Thanks to Anne Osbourn (John Innes Centre)

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About GARNet

GARNet represents the UK Arabidopsis researchers via a committee of elected members. It aims to make sure the UK Arabidopsis community remains competitive and productive at the national and international level by helping researchers make the best use of available funding, tools and resources.
www.garnetcommunity.org.uk

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Delegate Information

Meeting Location

An Introduction to Opportunities in Plant Synthetic Biology will be held in the Exchange Building on the University of Nottingham's Jubilee Campus. All the talks will be in the Lecture Theatre in the Exchange Building.

Travel by car: For satellite navigation the postcode for Jubilee is NG8 1BB. Parking is available in pay and display carparks.

Travel by train: The nearest train station to Jubilee Campus is Nottingham.

Conference badges

Conference participants are required to wear name badges at all times, for proof of registration, security purposes, and catering identification.

The coloured dot on your badge indicates the group you will be in for the breakout discussions.

Accomodation

Accomodation has been arranged at the National College for School Leadership (NCSL), which is situated on the Jubilee Campus. A map is provided on the next page showing the NCSL and the Exchange Building.

Conference dinner

The conference dinner is at the NCSL, which is situated on the Jubilee Campus. A map is provided on the next page showing the route between the Exchange Building and the NCSL.

Catering

Lunches will be served in the atrium of the Computer Science building. Refreshments will be served in the atrium of the Exchange building during tea and coffee breaks. Vegetarian options are labelled for your convenience. Please note that meat is not halal.

Internet access

Please ask at reception for a password to access the wireless network.

Twitter

If you are tweeting about the meeting, please use the hashtag #plantsynbio


Jubilee Campus
& University of Nottingham Innovation Park (UNIP)
MAIN ENTRANCE
24hr Security Gatehouse
Wollaton Rd.
Crown Island
Western Boulevard A6514
Wollaton Road A609
Ilkeston Road A609
To city centre
Melton Hall
PD
G
SC
The University of Nottingham
Jubilee Campus
Wollaton Road
Nottingham
NG8 1BB
University of Nottingham
Innovation Park
Sir Colin Campbell Building
Triumph Road
Nottingham
NG7 2TU
Middleton Boulevard A6514
To University Park Campus (0.5 miles)
Business School North
The Exchange
The Sir Harry & Lady Djanogly Learning Resource Centre
Computer Science
The Atrium
The Dearing Building
Southwell Hall
Newark Hall
International House
Business School South
Auditorium
Amenities Building
Sports Centre
Railway
Triumph Road
B
IPD
National College entrance
Sir Colin Campbell Building
National College for School Leadership (NCSL)
Sports ground
Tennis courts
N
0 metres 100
Pedestrian/cycle route to Jubilee Campus
To city centre
Pedestrian/cycle route to University Park Campus (0.5 miles)
To KMC Campus (1 mile)
Derby Road A6200
To University Park Campus
Academic schools and departments
Contemporary Chinese Studies 9
Computer Science 4
Civil Engineering 13
Education 6/15
International Office 10
Nottingham University Business School 1/7/10/14
Other services
Banks/Retail 2
Cafes 2/5/7/11/30
Faith/Prayer rooms 11
Graduate Centre 11
Libraries 3/7
Sports 30
Student Services Centre 11
Students' Union 11
University of Nottingham Innovation Park
Aerospace Technology Centre 18
Energy Technology Building 16
Institute of Mental Health 17
Nottingham Geospatial Building 13
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Halls of residence
Other services
University of Nottingham Innovation Park (UNIP)
University of Nottingham Innovation Park (UNIP) boundary
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Pay & Display visitor parking
UNIP Pay & Display visitor parking
Blue-badge parking
Gatehouse
Barrier-access control
Secure cycle parking
Hopper bus stop
Public bus stop
Public/Hopper bus stop
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Meeting Programme

Tuesday 21 May

09:30	Registration	Exchange Atrium
10:00	Jim Murray (University of Cardiff) Welcome and introduction	Lecture theatre

Session 1: What is synthetic biology, and what can it be used for?

10:15	Jim Haseloff (University of Cambridge) Engineering plant form
10:40	June Medford (Colorado State) Rewiring a plant and digital-like controls
11:05	Andy Boyce (Biotechnology & Biological Sciences Research Council) Research Council strategy and funding for synthetic biology
11:30	Belinda Clarke (Technology Strategy Board) Funding new frontiers in synthetic biology

Session 2: From molecules to cells and circuits

11:55	Dek Woolfson (University of Bristol) Generating and applying toolkits of *de novo* peptide components for synthetic biology	
12:20	**Lunch**	**Computer Science Atrium**
13:15	Cameron Alexander (University of Nottingham) Synthetic polymers – new containers and communication materials for synthetic biology	
13:40	Lee Cronin (University of Glasgow) Bottom up meets top down: From inorganic biology to synthetic biology manipulations in 3D printed wet-ware	
14:05	Martin Howard (John Innes Centre) Implementation of analogue arithmetic circuitry in plants	
14:30	Anne Osbourn (John Innes Centre) Making new molecules	
14:55	Rob Edwards (University of York; FERA) Plant synthetic biology: A new platform for industrial biotechnology?	

Session 3: Plant synthetic biology

Lecture theatre

15:20	Chloe Singleton (University of Exeter) Synthetic metabolons	
15:45	**Afternoon tea**	**Exchange Atrium**
16:05	Giles Oldroyd (John Innes Centre) Redesigning the symbiotic signalling pathway for rhizobial recognition	
16:30	Sebastian Schornack (Sainsbury Laboratory Cambridge) Targeted variation of genomes using TAL effectors	
16:55	Breakout groups: What can plants do for synthetic biology?	Various locations
19:30	Dinner at the National College for School Leadership	

Meeting Programme

Wednesday 22 May

08:45	**Tea and coffee**	**Exchange Atrium**
Session 4: Synthetic biology tools		Lecture theatre
09:00	Susan Rosser (University of Glasgow) Recombinases as tools for synthetic biology	
09:25	George Lomonossoff (John Innes Centre) eVLPs for plant synthetic biology	
09:50	Tom Ellis (Imperial College London) Assembling designer genomes	
10:15	Sylvestre Marillonnet (Icon Genetics) Developing tools for synthetic biology: Golden Gate Cloning and the MoClo System	
10:40	Jim Ajioka (University of Cambridge) A guide to Gibson assembly	
11:05	Coffee break	**Exchange Atrium**
11:30	Breakout sessions to discuss future community needs	Various locations
12:30	**Lunch**	**Computer Science Atrium**
13:30	Feedback from breakout groups	Lecture theatre
14:00	Claire Marris (Kings College London) Responsible Research and Innovation for Synthetic Biology	
14:25	Alistair Elfick (University of Edinburgh) iGEM	
14:50	Natalio Krasnogor (University of Nottingham) Computational tools for rapid model prototyping in synthetic biology	
15:15	Jim Haseloff (University of Cambridge) PlantFab registry of DNA parts for plants	
15:40	Richard Kitney (Imperial College London) Foundational Resources from cSynBi	
16:05	Guy-Bart Stan (Imperial College London) Taking a forward-engineering approach to the design of synthetic biology systems?	
16:30	**Close**	

Breakout Sessions Information

Your breakout groups are the same for the two discussion sessions. The dots on your badges represent the group you will be in. The groups are also labelled in the delegate list. Your group chair, rapporteur, and meeting location is given in the table below.

	Chair	Rapporteur	Location
Red	Andrew Spicer	TBC	
Green	Rob Edwards	Tom Ellis	
Blue	Anne Osbourn	Dek Woolfson	
Yellow	Susan Rosser	Jim Murray	
White	Giles Oldroyd	Ruth Bastow	

Breakout Session 1: What can synthetic biology do for plants?
Tuesday
16:55 Discussion in breakout groups
18:00 Feedback
18:30 Finish

1. What are the benefits of undertaking synthetic biology in plants?
2. What can plants contribute to synthetic biology?
3. If you were not limited by technology or resources, what new plants or plant products would you construct using synthetic biology approaches?
4. Which plant system(s) would provide a useful starting point for synthetic biology research?
5. What barriers would need to be overcome in order to carry out the projects outlined above?

Breakout Session 2: A plant synthetic biology community
Wednesday
11:30 Discussion in breakout groups
12:30 Lunch
13:30 Feedback

1. What current tools and resources exist to support plant synthetic biology?
2. What new tools and community resources are needed to allow plant researchers to make progress in this new sphere?
3. To what extent is the UK plant science community well placed to take advantage of the current opportunities in synthetic biology? What are the current barriers?
4. If there was an initiative to bring together a plant synthetic biology community, who should it include and what purpose would it serve?
5. Should such a community be limited to plant science, or should it be linked to communities that are already beginning to emerge in microbial or other areas?

Speaker Abstracts

Engineering plant form

Jim Haseloff

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Synthetic biology is an emerging field that employs engineering principles for constructing genetic systems. The approach is based on the use of well characterised and reusable components, and numerical models for the design of biological circuits – in a way that has become routine in other fields of engineering. Synthetic biology is providing a conceptual and practical framework for the systematic engineering of gene expression and behaviour in microbes, facilitating the design of novel regulatory networks, including synthetic oscillators, switches, logic gates, intercellular signaling systems and metabolic pathways. Synthetic biology approaches also show great potential for the engineering of multicellular systems. (1) The greatest diversity of cell types and biochemical specialisation is found in multicellular systems, (2) the molecular basis of cell fate determination is increasingly well understood, and (3) it is feasible to consider creating new tissues or organs with specialized biosynthetic or storage functions by remodelling the distribution of existing cell types. Of all multicellular systems, plants are the obvious first target for this type of approach. Plants possess indeterminate and modular body plans, have a wide spectrum of biosynthetic activities, can be genetically manipulated, and are widely used in crop systems for production of biomass, food, polymers, drugs and fuels.

Current GM crops generally possess new traits conferred by single genes, and expression results in the production of a new metabolic or regulatory activity within the context of normal development. However, cultivated plant varieties often have enlarged flowers, fruit organs or seed, and are morphologically very different from their wild-type ancestors. Recent genetic studies have provided detail of the molecular processes underlying plant development. The next generation of transgenic crops will contain small gene networks that confer self-organizing properties, with the ability to reshape patterns of plant metabolism and growth, and the prospect of producing neomorphic structures suited to bio production.

Morphogenesis is a cellular process, driven by interplay between gene expression and a growing network of cell interactions. We have developed a battery of microscopic and genetic tools to allow clear visualization of individual cells inside living plant tissues and have the means to manipulate them. These techniques are well suited to study of simple experimental systems such as the lower plant *Marchantia polymorpha*. Many of the molecular genetic tools that have been developed in Arabidopsis can be transferred to Marchantia. This type of simple system is becoming increasingly important for plant morphogenetic studies.

Rewiring a plant and digital-like controls

June Medford

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Plant synthetic biology has the potential to enable sustainable human existence on earth. That is an audacious statement. Yet, prior to the late 1800s most humans derived their food, materials and shelters from biological organisms. In the early 1900s chemists produced materials such as plastics that revolutionized societies. A revolution to produce sustainable products and materials is upon us. Plants are outstanding platforms for synthetic biology and can be designed to serve humanity and produced renewable and environmentally friendly materials and shelters. For example, we have designed a synthetic biological input -output system that enables external control of a trait of interest in plants. It is orthogonal to plant function allowing widespread adaptation. Our circuits are highly modular and can be used to control production of materials or produce plants that enable ordinary people to know about dangerous substances such as pollutants in their environments. We have tuned our traits with 'logic circuits' to provide amplification, memory and digital-like controls to transcriptional traits. These logic circuits are also being applied to bioenergy traits to enable at-will production of energy materials. To develop the promise of plant synthetic biology and produce sustainable existence on earth will require efforts of many and willingness to see beyond current limits.

Research Council strategy and funding for synthetic biology

Andy Boyce

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The UK Research Council were amongst the first funders worldwide to recognise the importance of synthetic biology. Since 2007, they have been supporting the field through networking awards, public dialogue, training, international projects and a number of large scale funding programmes.

BBSRC and EPSRC have committed over £80M to the area and were active in the development of the UK Synthetic Biology Roadmap, which provides a framework for UK policy in this area going forward. They continue to actively work with the other Research Councils, the Technology Strategy Board and our international partners on the next stage of support for synthetic biology.

Funding new frontiers in synthetic biology

Belinda Clarke

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The extensive and ongoing support for the UK's academic synthetic biology industry is manifesting itself in commercial reality through start-ups and spin outs. The Technology Strategy Board is working with research council co-funders and the Synthetic Biology Leadership Council to help technologies through potential barriers to commercialisation to support such activities and to help small companies grow. The size and shape of the UK's synthetic biology community will be discussed, with potential new funding streams under consideration.

Protein design and engineering in synthetic biology: Towards new materials and micro-compartments

Dek Woolfson

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Synthetic biology presents challenges for fundamental and applied science, and engineering. It asks if we understand enough about biology to modularise and standardise into parts; and, then, if such parts can be designed, engineered and combined in new ways to achieve new functions. True, we can engineer biological systems now, and success is being achieved in the area; and we should continue this empirical, ambitious and sometimes even fanciful approach to the design and engineering of biology. However, I believe that big leaps will come through improved understanding of how biological systems are built and how they function.

This need for further understanding is certainly the case when it comes to designing and engineering proteins where we still haven't cracked the protein-folding code, and we are a long way off designing or even tinkering with protein function, for example enzyme activity, in predictable ways. That said, if we do not try we will never know how far this can be pushed. In addition, the synthetic-biology approach inspires us to dream up, and put into place ever more ambitious design and engineering goals. In my view, this new approach brings a breath of fresh air to the protein-design field.

I shall outline one view of what the field of synthetic biology is and might become in its broadest sense; and then how protein designers and engineers might benefit from and contribute to this exciting new area. I will illustrate this with some recent examples from my own lab's research, including designs for new biomaterials and self-assembled nanoscale compartments with potential applications in medicine and biotechnology.

References

EHC Bromley, K Channon, E Moutevelis, and DN Woolfson (2008) Peptide and protein building blocks for synthetic biology: From programming biomolecules to self-organized biomolecular systems. ACS Chemical Biology 3, 38-50

NR Zaccai, B Chi, AR Thomson, AL Boyle, GJ Bartlett, M Bruning, N Linden, RB Sessions, PJ Booth, RL Brady and DN Woolfson (2011) A de novo peptide hexamer with a mutable channel. Nature Chemical Biology 7, 935-941

JM Fletcher, AL Boyle, M Bruning, GJ Bartlett, TL Vincent, NR Zaccai, CT Armstrong, EHC Bromley, PJ Booth, RL Brady, AR Thomson, and DN Woolfson (2012) A Basis Set of de Novo Coiled-Coil Peptide Oligomers for Rational Protein Design and Synthetic Biology. ACS Synthetic Biology 1, 240–250

JM Fletcher, RL Harniman, FRH Barnes, AL Boyle, A Collins, J Mantell, TH Sharp, M Antognozzi, PJ Booth, N Linden, MJ Miles, RB Sessions, P Verkade and DN Woolfson (2013) Self-assembling Cages from Coiled-coil Peptide Modules. Science In Press (DOI: 10.1126/science.1233936)

Synthetic polymers – new containers and communication materials for synthetic biology

Cameron Alexander

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Synthetic biology covers a variety of scientific disciplines and offers many opportunities for biomedical technologies and applications. In particular, the idea of an 'intelligent' therapeutic, activated to release a drug on recognition of an early disease signal is very appealing from a clinical perspective. However, in order to develop such a specifically bio-activated device, a number of technologies need to be coupled together, many of which are in their infancy.

In Nottingham, we have been working, as part of the EPSRC-funded 'Chell' consortium, on synthetic container components which might act as primitive mimics of an intelligent synthetic biology. Our goal is to generate complexity in function from abiotic parts, each of which has a specific function that can be related to a real biological cell. We aim to couple chemical information, a simple metabolism model, and a container to gate information flow, into a 'Turing test' experiment[1, 2] such that we evaluate our chemical cell ('Chell') against information flow and signaling with real bacterial cells.

The talk will accordingly focus on our latest results in which we have been developing polymers and containers that interfere with bacterial Quorum Sensing (QS) systems.[3] We will show that polymers can feedback into a range bacterial QS networks, both through signal capture and cell recognition. The data suggests polymer containers may have applications not just as 'intelligent agents' against bacterial infections but may also be used as components in synthetic biology circuits.

References

1. L Cronin, N Krasnogor, BG Davis, C Alexander, N Robertson, JHG Steinke, SLM Schroeder, AN Khlobystov, G Cooper, PM Gardner, P Siepmann, BJ Whitaker, D Marsh (2006) The imitation game - a computational chemical approach to recognizing life. Nature Biotechnology 24, 1203-1206

2. G Pasparakis, N Krasnogor, L Cronin, BG Davis, C Alexander (2010) Controlled polymer synthesis-from biomimicry towards synthetic biology. Chemical Society Reviews 39, 286-300

3. X Xue, G Pasparakis, N Halliday, K Winzer, SM Howdle, CJ Cramphorn, NR Cameron, PM Gardner, BG Davis, F Fernandez-Trillo, C Alexander, (2011) Synthetic Polymers for Simultaneous Bacterial Sequestration and Quorum Sense Interference. Angewandte Chemie-International Edition 50, 9852-9856.

Bottom up meets top down: From inorganic biology to synthetic biology manipulations in 3D printed wet-ware

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In our laboratory we have been developing new approaches to discover the 'transition-to-evolvability' in chemistry. This is because if we can discover or engineer an abiotic system that can evolve (we could define this as an inorganic chemical cell -iCHELL) we might be able to suggest that synthetic biology can exist in many chemical forms, of which the terrestrial biology found on planet earth is one subset. It could even help us establish the idea that evolvability is the key signature that defines living from non-living systems. This problem is rather vast since our aim is to compress a planet sized reaction vessel and a 400 M year run-time into a laboratory over a few years! Not only does this extraordinary problem require new radical chemical approaches,[1] it also requires the development of some radical new technological solutions.[2,3] In this talk I will cover both aspects with an emphasis on how some of our new approaches can be applied to both 'inorganic' and 'organic' synthetic biology.

1. GJT Cooper, PJ Kitson, R Winter, M Zagnoni, D-L. Long, L. Cronin (2011) Modular Redox Active Inorganic Chemical Cells: iCHELLs. Angew. Chem. Int. Ed. 50, 10373. DOI: 10.1002/anie.201105068

2. CJ Richmond, HN Miras, A R de la Oliva, H Zang, V Sans, L Paramonov, C Makatsoris, R Inglis, EK Brechin, D-L Long, L Cronin (2012) A flow-system array for the discovery and scale up of inorganic clusters Nature Chem. 4, 1037-1043

3. MD Symes, PJ Kitson, J Yan, CJ Richmond, GJT Cooper, RW Bowman, T Vilbrandt, L Cronin (2012) Integrated 3D printed reactionware for chemical synthesis and analysis. Nature Chem. 4, 349-354

Implementation of analogue arithmetic circuitry in plants

Martin Howard

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Starch reserves that accumulate in Arabidopsis leaves during the day decrease approximately linearly with time at night to support metabolism and growth. The rate of decrease is adjusted to accommodate variation in the time of darkness and the level of starch, such that reserves last almost precisely until dawn. We show that generation of these dynamics requires an arithmetic division computation between the starch levels and the expected time to dawn thereby allowing calculation of the appropriate starch degradation rate. This is a specific example of analogue computation in biology and we introduce two novel chemical kinetic models capable of implementing such analogue arithmetic division. Predictions from the arithmetic division hypothesis for starch degradation kinetics are then successfully tested in plants perturbed by a night-time light period or by mutations in starch degradation/circadian clock pathways. Our results are potentially relevant for any biological system dependent on a stored food reserve for survival over a predictable period of time.

Making new molecules

Anne Osbourn

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Plants produce a tremendous array of natural products, including medicines, flavours, fragrances, pigments and insecticides. The vast majority of this metabolic diversity is as yet untapped, despite its huge potential value for humankind. So far research into natural products for the development of drugs, antibiotics and other useful chemicals has tended to focus on microbes, where genome sequencing has revolutionised natural product discovery through mining for gene clusters for new metabolic pathways. Identifying novel natural product pathways in plants is extremely difficult because plant genomes are much larger and more complex than those of microbes. However, the recent discovery that genes for some types of plant natural product pathways are organised as physical clusters is now enabling systematic mining of plant genomes in the quest for new pathways and chemistries. Improved understanding of the genomic organization of different types of specialized metabolic pathways will shed light on the mechanisms underpinning pathway and genome evolution. It will further open up unprecedented opportunities for exploiting Nature's chemical toolkit by providing grist for the synthetic biology mill.

Plant synthetic biology: a new platform for industrial biotechnology?

Rob Edwards

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Thirty years after the development of GM plants we are now set to move into a new era of recombinant crop technology through the application of synthetic biology to engineer new and complex input and output traits. In particular synthetic biology in plants should not be considered to be just a form of transgene pyramiding, but rather the directed design of completely new metabolic pathways, physiology and development. It is also clear from the recent cross research council funded public dialogue on the synthetic biology that some applications for the technology will be more likely to be welcomed than others. For example engineering plants for medical applications would be likely to be better received than improving crops for food characteristics such as process ability or nutritional value. It is therefore important that in embarking on using plants as the subject of synthetic biology that applications which qualify as being of 'public good', but avoid legacy issues of GM food are adopted from the outset. In this presentation the potential benefits and risks of using an overt synthetic biology approach to improve plants for industrial (non-food) applications will be considered. This will include identifying the types of desirable input and output traits which might be targeted, the frameworks for assessing such products in a policy and economic framework and the associated academic and technical challenges.

Synthetic metabolons: application to improvement of photosynthesis

Chloe Singleton and Nick Smirnoff

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Aggregation of enzymes involved in various metabolic pathways into complexes (metabolons) has been proposed to provide benefits including substrate channelling (direct transfer of the product of one enzyme to the active site of the next) or increased local substrate concentration. This may increase flux or decrease the concentration of enzymes required to maintain a given flux. Release of potentially toxic intermediates may also be minimised. Metabolons form by protein-protein interaction, attachment to membranes or attachment to the cytoskeleton. However naturally occurring metabolons may be difficult to detect because interactions may be weak or transient. In plants there is evidence for metabolon formation in flavonoid biosynthesis and some other pathways. In prokaryotes, metabolons also arise when enzymes are enclosed in protein-coated microcompartments, the carboxysome in cyanobacteria being the most well-known.

Synthetic metabolons, in which enzymes are scaffolded to synthetic proteins or nucleic acids, or encapsulated into microcompartments, may be of benefit to metabolic engineering efforts and are being explored in microorganisms. The application of this approach to improving photosynthesis will be discussed and early steps in making a synthetic CO_2 fixing complex containing ribulose bisphosphate carboxylase-oxygenase (Rubisco) and carbonic anhydrase will be presented.

Engineering nitrogen fixation in cereals

Giles Oldroyd

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The ability to take up mineral nutrients, particularly nitrogen and phosphorus, is generally the major limitation to plant growth. Because of this farmers apply nitrogen and phosphorus through fertiliser application to promote crop growth. Sustained yields are dependent on this fertiliser application, but it comes at a high price, both in the cost of the fertiliser and the environmental damage that results from its use. A number of plant species have evolved beneficial interactions with micro-organisms that facilitate the uptake of these nutrients. Legumes form symbiotic interactions with mycorrhizal fungi that facilitate phosphate uptake and with rhizobial bacteria that provide the plant with a source of nitrogen. The establishment of these symbioses involves a molecular communication between the plant and the symbiotic micro-organisms in the soil. Mycorrhizal fungi and rhizobial bacteria release signals that are recognised by the host plant and lead to developmental changes associated with the accommodation of the symbionts. Genetic dissection in the legume *Medicago truncatula* has defined the signalling pathways involved in these symbioses. A number of the genes required for the mycorrhizal interaction are also necessary for the rhizobial interaction, indicating a conserved symbiosis signalling pathway. This implies that the evolution of nodulation involved the recruitment of a signalling pathway already functioning in mycorrhizal signalling. This signalling pathway is present in most plant species, including cereals suggesting that engineering the perception of rhizobial bacteria in cereals is simplified and requires an understanding of the legume specific components that activate and are activated by the common symbiosis signalling pathway. We are in the process of engineering this signalling pathway in cereals to promote the recognition of rhizobial bacteria as the first step in engineering biological nitrogen fixation into cereal crops.

Targeted variation of genomes using TAL effectors

Sebastian Schornack

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The last years have seen a growing demand for genome engineering. Transcription activator-like (TAL) effectors from plant pathogenic bacteria which induce expression of plant host genes have become the focus of intense activity. These effectors bind to DNA using a code that specifies association of amino acid pairs in repeat units to particular DNA bases in a one-to-one fashion. This code can be used to design novel synthetic DNA-binding domains for targeted genome variation. The unique DNA recognition modality of TAL effectors has been rapidly and widely exploited in biotechnology to design and build synthetic proteins comprised of DNA-binding domains based on TAL effector repeats fused to various functional elements, including nucleases, recombinases, activation domains, and repressor domains. TAL effector-based tools have successfully been implemented in a growing list of organisms that spans single cell organisms, mono- and dicotyledonous plants, insects, fish and mammals. I will give an overview about the principle of targeted variation using TAL effectors, compare them with other existing systems and emphasize some community resources and recent applications.

Recombinases as tools for synthetic biology

Susan Rosser

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One major target in synthetic biology is the creation of genetically modified organisms, to produce valuable chemical substances economically, in high yield and with low environmental impact, or to carry out beneficial chemical transformations. To create these organisms, it is often necessary to introduce a set of new genes and assemble them in specified positions within the organism's genome. The genetic techniques currently available for this 'assembly' task are still inadequate, and gene assembly is considered to be a serious bottleneck in the work leading to the development of useful microorganisms. The first main aim of our research programme is to establish a sophisticated new methodology for this gene assembly process which will achieve a step- change in the speed and efficiency of creating new microorganism strains. For this purpose we have adapted a remarkable group of bacterial recombinases whose natural task is to carry out this kind of genetic rearrangement but which have hitherto been underused as tools for synthetic biology. We have designed rapid, robust and efficient ways of making gene cassettes that can be recombined in to specified positions in DNA. By doing this we can assemble collections of genes to order within a particular microorganism. Furthermore we can choose where to place the genes and in what order, and replace any individual parts with different versions. This permits much easier optimization of complex genetic systems than is currently possible. Using our new methods we intend to engineer microbial cells to make useful products e.g. next-generation biofuels, fine and platform chemicals.

Empty virus-like particles (eVLPs) for plant synthetic biology

Keith Saunders, Alaa AA Aljabali, Pooja Saxena, Yulia Meshcheriakova, David Evans and **George P Lomonossoff**

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Particles of the icosahedral bipartite RNA plant virus, Cowpea mosaic virus (CPMV), have been modified both genetically and chemically to allow the display of a wide variety of chemical moieties, including peptides, fluorescent dyes, organo-metallic complexes, sugars and polymers on the outer virus surface. Until recently, the particles, either wild-type or genetically modified, were exclusively produced by infecting plants with the viral genome. This approach has two principle disadvantages: (1) the range of modifications which can be introduced is limited by the need to maintain virus viability and (2) the majority (90%) of the particles contain the viral genomic RNA meaning that they retain their infectivity and cannot be loaded with heterologous material. To address these issues, we have made use of the recently developed CPMV-*HT* system transient expression system to create synthetic empty virus-like particles (eVLPs) *in planta*. Such eVLPs can be produced in amounts similar to the levels of RNA-containing virus produced via infection. However, they are entirely non-infectious and can be loaded with a variety of heterologous molecules. Furthermore, they can potentially be subjected to far more extensive genetic modification, including to the inner particle surface, than is possible with RNA-containing particles, greatly increasing the potential uses of CPMV-based technologies in synthetic biology.

Assembling designer genomes

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Synthetic biology seeks to understand and derive value from biology via its re-design and synthesis using engineering principles. Towards this goal, our lab has been working on foundational tools to enable this for a variety of organisms and applying these tools to projects on metabolism, logic, biosensing and genome engineering. In this talk, I will first present our work on a modular, combinatorial strategy for DNA assembly that arranges genes and gene libraries into pathways and networks and uses custom software tools to enable this. I will then discuss our lab's move into genome synthesis, where we have joined the global Sc2.0 project to synthesise and assemble a human-designed version of the *S. cerevisiae* genome. Our lab is working on assembling chromosome XI for this project, and facing the challenges of large DNA assembly projects in an era of cheap DNA.

Developing tools for synthetic biology: Golden Gate Cloning and the MoClo System

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A basic requirement for synthetic biology is the availability of efficient DNA assembly methods. We have developed a modular cloning system that allows assembly of multigene constructs using a series of one-pot assembly reactions. At the base of the system, libraries of basic parts containing basic genetic elements such as promoters, coding sequences and terminators are cloned as standard level 0 modules. Basic parts are assembled into transcription units using a first assembly reaction. Transcription units are then assembled in multigene constructs using one or several successive assembly reactions. As an example, a construct containing 27 transcription units was assembled from 81 basic modules in three cloning steps. This modular cloning system can be used for assembly of multigene constructs for expression in either eukaryotes or prokaryotes and should be useful for applications such as gene stacking, metabolic engineering and synthetic biology.

A guide to Gibson assembly

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Responsible research and innovation for synthetic biology

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One of the interesting novelties about the field of synthetic biology is that it has actively tried to engage with social and ethical issues early on. More than 40 reports on the social, legal, environmental and ethical issues related to synthetic biology have already been produced, and social scientists were enrolled early on to help identify and address these issues. In the UK, this has often been a requirement from research funding councils, and addressing such issues is increasingly framed in terms of encouraging "responsible research and innovation" (RRI). RRI is being promoted within the European Commission and will become a crosscutting issue for Horizon2020 funding programmes, and was identified as a key theme in the UK Synthetic Biology Roadmap published in July 2012. RRI can, has, and will be interpreted in many different ways, and promoted for different purposes. I will argue that RRI should aim to ensure that synthetic biology focuses on how to deliver benefits for the public good, and moreover that defining what counts as the 'public good' needs to involve deliberation with a wide range of different social groups and realms of expertise. Synthetic biology needs to move beyond concerns about public attitudes towards experimenting with methods to genuinely respond to societal concerns and tackle global challenges. RRI needs to be about transforming synthetic biology research and innovation, rather than about managing public acceptance.

iGEM

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Computational tools for rapid model prototyping in synthetic biology

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The conceptual cornerstone of synthetic biology is that methodologies commonly used to design and construct non-biological artefacts (e.g. computer programs, airplanes, bridges, etc) might also be mastered to create “designer” living entities. In particular, and notwithstanding that a biological substrate is very different than electronic computers, one would like to be able “program” the former with the same ease as one programs the later.

This talk presents progress being made in trying to develop an integrated computer aided design (CAD) suite for programming cellular behavior via rapid biomodel prototyping. Biomodels are formally specified using a domain specific programming language (InfoBiotics) that captures several layers of biological organization (colony level, cellular level, sub-cellular processes) and permits models reuse. An InfoBiotics program (i.e. a prototype biomodel exhibiting a designer phenotype) can be executed with state-of-the-art stochastic simulators and analysed via model checking techniques. Once the target phenotype is achieved *in silico*, the InfoBiotics program must be converted into well-defined DNA sequences ready for synthesis. Currently, this process is knowledge-intensive; our methodology automates (part of) this process by compiling the formal specifications into a detailed list of biological DNA parts. Usually, a compiled list of parts requires substantial optimisation (e.g. strengthening/weakening of binding sites, degradation rates, etc) in the wet lab. To formally capture this process, a domain specific programming language, DNALD, is used to program combinatorial DNA libraries.

Time permitting, I will briefly mention challenges and opportunities for computer scientists working in synthetic biology.

Registry of DNA parts for plants

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A formidable array of biochemical, biophysical and genetic techniques have been assembled for the description of biological systems, and this has given us methods for the comprehensive description of an organism's genome, gene expression patterns and metabolic activities. New imaging techniques allow us to monitor activities within living organisms and to precisely reconstruct cellular architecture. In addition advances in the technology of DNA synthesis and assembly have allowed the copying and reconstruction of entire bacterial chromosomes. This has raised the prospect of wholesale reprogramming of biological systems, or creation of new organisms. Unfortunately, the capacity for DNA synthesis has far outstripped our ability to design new or modified genetic systems on a similar scale.

The field of synthetic biology is based on the use of well characterised and reusable components and numerical models - for the design of biological circuits, in a way that has become routine in other fields of engineering. It is providing a conceptual and practical framework for the systematic engineering of biological systems. The emergence of these standards has provided a challenge to provide systems for improved information storage and access to DNA parts. We are exploring open source and low cost tools for handing DNA parts (www.plantfab.org), DNA assembly (www.gibthon.org) and modelling (www.cellmodeller.org), and will describe these.

Foundational resources for synthetic biology: A systematic design approach to synthetic biology

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Synthetic biology is a rapidly developing field, even through to consideration of the translation process from academia to industry. The presentation will consider systematic design in the context of the engineering principles of modularisation, standardisation and characterisation. On this basis it is possible to develop systematic design workflow. Examples will be given of this approach in relation to areas of application. The development of platform technology will be discussed, particularly with regard to information systems and the development of international standards.

A web-based information system which has been developed for synthetic biology will be described. This comprises a four layer model, the HTML layer, the communication layer, the application layer and the database layer. Part characterisation procedures will be described in the context of the information system. An important part of the information system comprises the incorporation of data and metadata from part characterisation. Two aspects of this will be discussed. First, how the system handles parts from other sources and Centres. Second, how parts are characterised using robotic techniques within our Centre at Imperial College. The need for international standards in synthetic biology will be discussed. Two specific standards will be described, these being DICOM-SB and SBOL – along with other aspects of standards, including standards for the physical assembly of DNA and the Standard European Vector Architecture (SEVA). The final part of the talk will describe the translation process in more detail, particularly in the light of the recent UK Government synthetic biology roadmap.

Taking a forward-engineering approach to the design of synthetic biology systems

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In this talk I will give a brief overview of some of the research activities in my group, the “Control Engineering Synthetic Biology” group at Imperial College London, where we focus our efforts on developing foundational forward-engineering tools to rigorously analyse, design and control synthetic gene circuits and cellular metabolism so as to endow engineered cells with novel functionalities. The tools and approaches that we take rely on principles drawn from Robust Optimal Control and Dynamical Systems theory, applied to Systems and Synthetic Biology problems. I will also briefly cover some of the current efforts that are currently made to develop communication standards allowing various computer-aided-design tools to exchange information and collaborate.